REMARKS

In the Action, the Examiner maintained the rejection of claims 1-17 and 22-28 and rejected new claims 31-33 under 35 USC §112, second paragraph, as assertedly being indefinite.

The Examiner also rejected claims 1-7, 13-17, 24, 27 and 31-32 under 35 USC §103(a) as assertedly obvious in view of Meinhardt (hereinafter "Meinhardt"), Butler et al. (Yeast 7:617-25, 1991) (hereinafter "Butler"), further in view of U.S. Patent 6,410,271 (hereinafter "the 271 patent").

Claims 22 and 23 were rejected as obvious in view of Meinhardt, Butler, the '271 patent and Monschau (Applied Envtl Microbiol 64: 4283-90, 1998) (hereinafter "Monschau").

Claim 28 was rejected as obvious in view of Meinhardt, Butler, the '271 patent and Jirholt (Gene 215:471-76, 1998) (hereinafter "Jirholt").

Reconsideration is requested in light of the following amendments and remarks.

I. Support for the Amendments to the Claims

Support for the amendment to the claims is found throughout the specification. For example, support for the amendment to claim 1 to recite yeast cells sensitive to the γ toxin is found at page 7, lines 15-31. Claim 6 has been amended to clarify the term unique recognition site. Claim 8 has been amended to clarify a limitation of the DNA recited in claim 2. The amendments include no new matter.

II. The rejection of claims 1-17 and 22-28 under 35 USC §112, second paragraph, as assertedly indefinite should be withdrawn

The Examiner variously rejected claims 1-17 and 22-28 under 35 USC §112, second paragraph, as assertedly indefinite, as follows.

Claim 1 was rejected as assertedly being indefinite for recitation of the term "suitable" e.g., suitable cells and suitable conditions. The term has been removed from the

claim and the claim amended to clarify the type of cells used in the method, thereby obviating the rejection.

Claims 6, 7, 14 and 24 were rejected as assertedly being indefinite for recitation of the phrase "unique recognition site." The Examiner alleged it is unclear what makes a recognition site unique. Claim 6 as amended recites "wherein said ... DNA sequence ... comprises at least one unique restriction enzyme recognition site that is unique for a given restriction enzyme." Claim 6 refers to DNA in which there may be more than one restriction enzyme which is specific for only one recognition site in said DNA sequence, i.e., implying that multiple unique recognition sites may be present. As described previously, the term "unique" is used in the sense of a single recognition site, such that one specific restriction enzyme will cut only once in a given DNA sequence. One of ordinary skill readily recognizes this property of a restriction enzyme toward DNA.

For example, a multiple cloning site, a typical component of cloning plasmids, provides a number of restriction sites which are unique within a short sequence stretch and allow for efficient integration of the target sequence into the desired site. Attached (Exhibit A, Hill et al., Yeast 2:163-67, 2004) is an exemplary abstract on a cloning vector stating that "all ten restriction sites of the multiple cloning region of pUC I8 including EcoRI, SacI, KpnI, SmaI, BamH1, XbaI, SbaI, Sall, PstI, SphI and HindIII are unique in YEp352; these sites are also unique in YEp35I except for EcoRI and KpnI, which occur twice" (Emphasis added). Thus, the term "unique" with respect to restriction enzymes and recognition sites is understood by one of ordinary skill in the art and the claim is clear as written.

Claim 8 was rejected as assertedly being indefinite for reading that the γ subunit is substituted by an antibody. Applicants submit there was a typographical error in claim 8 as filed, and claim 8 should have been dependent from claim 2. Claim 8 has been amended to depend from claim 2 and recite that the second DNA sequence is an antibody. As stated on page 3, lines 25-27 and page 4, lines 18-20, the target vector comprises a second DNA sequence which preferably encodes at least one protein region (claim 2) which is an antibody or a single chain antibody (claim 8).

Amendment to claim 8 obviates the rejection to claims 9, 10 and 25-26.

For the foregoing reasons, the rejection of the claims under 35 USC §112, second paragraph, should be withdrawn.

III. The rejection of claims 1-7, 13-17, 24, 27 and 31-32 under 35 USC §103(a) as assertedly obvious in view of Meinhardt, Butler and US 6,410,271 should be withdrawn

The Examiner rejected claims 1-7, 13-17, 24, 27 and 31-32 under 35 USC §103(a) as assertedly obvious in view of Meinhardt and Butler, further in view of the '271 patent. Applicants respectfully disagree.

The obviousness rejection over Meinhardt, Butler and the '271 patent should be withdrawn because the references, taken alone or in combination, do not teach all the recited claim elements, and, moreover, teach away from the present invention thereby lacking a motivation to combine the references to arrive at the present invention. Each of the cited documents teach use of positive selection cloning for selecting for homologous recombination, and fail to suggest any negative selection cloning method is useful for generating a randomized library, and certainly do not suggest the γ toxin subunit as a negative selection marker. Thus, all the elements of the claims are not found in the cited art, and the art provides no motivation to arrive at the present invention since the art teaches away from the methods disclosed herein. Further, the claimed methods provide unexpected results compared to prior art methods.

i) The art does not disclose all the elements of the claims nor would the elements be obvious from the cited art

Meinhardt neither discloses nor suggests use of the γ toxin as a negative selection marker such that excision of this gene results in selection for positive homologous recombination, "thereby abolishing expression" of the γ subunit with the donor DNA, nor suggests using a γ toxin sensitive cell line as recited in the claims. Meinhardt discloses use of a target vector having the linear plasmid pGKL1 of *K. lactis* comprising the α, β and γ subunits of the killer toxin gene. Meinhardt discloses homologous recombination in a suitable γ toxin-resistant cell, i.e., *K. lactis*, wherein the α and β subunits of the killer toxin gene are replaced by the LEU2 and Aph selection markers, and in the resulting vector, the donor genes LEU2 and Aph and the γ toxin gene (in ORF4) are present (Figure 2 of

Meinhardt). Thus, in Meinhardt the selection markers are the donor sequences and not the target DNA sequence as in the present method, and the donor sequences do not excise the γ toxin. Further, Meinhardt is silent about methods to generate random gene libraries, and therefore there is no suggestion to use a negative selection growth marker to make expression libraries by homologous recombination.

Butler neither discloses nor suggests a method of making a randomized gene library, nor discloses or suggests that the γ toxin is useful as a negative selection marker. Butler discloses that target cells (*S. cerevisiae,* i.e., cells sensitive to the γ toxin) that have been transformed with a plasmid carrying the γ toxin gene (pGKL1 ORF4) are unable to form colonies (p 617, 2nd col.). The goal of Butler is to express the γ toxin in the host cell, in contrast to the present methods which require excision of the γ toxin.

The '271 patent relates to the production of expression libraries and generation of fusion proteins using homologous recombination in yeast. The '271 patent discloses only the use of positive selection markers, such as nutritional reporter genes, for selecting for positive homologous recombination (col. 40, lines 36-48). The '271 patent neither discloses nor suggests that a negative selection system as taught in the present application is useful in generating a random gene library, and certainly does not suggest using the γ toxin gene as a negative selection marker.

As noted above, none of the cited art teaches that the γ toxin gene is useful as a negative selection marker such that excision of the γ toxin by the donor DNA signifies successful homologous recombination of the donor and target DNA, and abolishes expression of the γ subunit. Further, none of the art discloses use of a γ toxin-sensitive cell for homologous recombination when using the γ toxin gene as a negative selection marker. Moreover, these elements would not be obvious from the cited art since none of the art describes any negative selection methods, and certainly does not teach use of the γ subunit as a negative selection marker. It would not have been obvious to a person of ordinary skill to carry out methods that are contradictory to those described in the art, such as switching from positive selection cloning to negative selection cloning, to arrive at the present invention.

Because none of the art discloses or suggests use of the *K. lactis* γ toxin gene as a negative selection marker for homologous recombination to create a randomized gene

library, the Examiner has failed to establish a *prima facie* case of obviousness and the rejection of claims 1-7, 13-17, 24, 27 and 31-32 under 35 USC §103(a) as obvious in view of Meinhardt, Butler and the '271 patent should be withdrawn.

ii) The art teaches away from the present invention, and therefore provides no motivation to combine the references

Even if *arguendo*, all the claim elements were present in the cited art, Meinhardt, Butler and the '271 patent, taken alone or in combination, teach away from the present methods, and as such provide no motivation to one of ordinary skill to combine the disclosures to carry out the methods of the invention. Each of the cited art disclosures teach positive selection cloning and make no suggestion to modify the cloning techniques to negative selection as used herein.

The method of Meinhardt is not useful for generating a randomized gene library given the use of the positive selection markers as donor sequences. Meinhardt discloses heterologous gene expression from a linear plasmid located in the cytoplasm and screening for homologous recombination of a cytoplasmatically located (linear) plasmid (see p. 319, 1st col., 2nd paragraph). One of ordinary skill constructing randomized gene libraries would not have modified the method of Meinhardt to arrive at the present invention since Meinhardt suggests the use of a direct positive selection marker to signify successful recombination and relates to homologous recombination in the cytoplasm as opposed to the homologous recombination in the present methods, which takes place in the nucleus. Meinhardt teaches scientific methods contrary to the methods of the invention (e.g., positive not negative selection), and would not have motivated one of ordinary skill to modify Meinhardt to arrive at the present invention.

Additionally, like Meinhardt, Butler teaches away from the present invention and therefore would not have motivated one of ordinary skill to modify Butler to arrive at the present invention. Butler utilizes γ toxin expressed on a plasmid and states that "unlike the arrest promoted by native toxin, intracellular expression of the γ-subunit was not accompanied by loss of viability of the arrested cells and its effects were fully reversible" (page 623, 2nd col., 2nd paragraph). Butler also identified mutants that are resistant to the γ-toxin induced G1 arrest (page 620, 2nd col.). Using a plasmid of this nature was

counterproductive in generating a randomized gene library by negative selection since spontaneous mutants resistant to the γ-toxin increase detection of false positives and interfere with screening and selection of the positive clones. One of ordinary skill would have recognized from Butler that a plasmid having the γ subunit expressed intracellularly can lose its growth arrest ability in culture, which would lead to inconsistent selection of clones and difficulty in consistent library production. Thus, one of ordinary skill in the art would not have been motivated to use or modify the method of Butler to carry out the present methods.

US 6,410,271 is silent about the use of a negative growth selection marker to improve the process of library generation, and suggests that nutritional reporter genes and positive selection are preferred for generating random gene libraries. Thus, the '271 patent teaches one of ordinary skill to use positive selection cloning as opposed to the negative selection cloning in the present methods.

There would have been no motivation to use a negative selection marker as in the present invention when each of Meinhardt, Butler and the '271 patent use positive selection, i.e., the use of the LEU2, aph and/or URA3 markers, to confirm homologous recombination. All of the cited art teaches use of positive selection markers, thereby teaching away from using the negative selection method described in the specification. One of ordinary skill would not have been motivated by the cited art to use a negative selection marker for cloning libraries when none of the references suggest any negative selection technique, and certainly do not suggest using the γ subunit as a negative selection marker.

Combining the teachings of Meinhardt and Butler as suggested by the Examiner would have led to use of the pGKL1 plasmid of Meinhardt comprising the α, β and γ subunit and the donor sequence having positive selection markers transfected into a toxin-sensitive yeast cell of Butler. Homologous recombination in these cells would have the selection markers in place of the α and β subunits, but express the γ toxin subunit gene in a γ toxin-sensitive yeast cell, leading to cell death. Thus, the combination of Meinhardt and Butler suggested by the Examiner leads to inoperable cloning techniques.

Moreover, in the present case, the combination of art proposed by the examiner would change the principle of operation of the art disclosed methods. If the proposed modification or combination of the prior art would change the principle of

operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. See MPEP 2143.01 citing *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959). Reliance on the cited art to arrive at the present invention required modifying the positive selection cloning in each of Meinhardt, Butler and the '271 patent by redesigning the cloning elements and changing the basic principle under which the art was designed to operate, thereby arriving at negative selection cloning. Thus, the cited art is insufficient to establish a *prima facie* case of obviousness.

iii) The specification provides unexpected results over the cited art

None of Butler, Meinhardt or the '271 patent, taken alone or in combination, recognized that use of the negative selection method described herein surprisingly significantly reduces background clones (e.g. false positives) and improves cloning efficiency when generating a randomized gene library.

For example, the method described in the '271 patent allows for generation of libraries where a minimum of 90% recombinant clones are preferred, i.e., there is a margin for 10% background (see col. 32, lines 38-39). Although this may be suitable for a simple cloning event, it is not suitable for library randomization, e.g., when generating a library having improved binding affinity of an scFv for a given epitope. For example, background clones are problematic in the randomization of an antibody light chain complementarity determining region CDR3 of a weak binding antibody and selecting for randomized CDR3 peptides with increased affinity (see page 11, lines 26-34 of the specification). The background clones in such a situation bind the epitope weakly and can interfere with the selection of improved binders by giving false positives (see page 11, lines 26-34).

Applicants discovered that the claimed selection method decreased the background of the homologous recombination and improved cloning efficiency, thereby improving expression of the randomized gene library. Page 16, lines 31-35, of the specification discloses that use of the γ subunit as a negative selection marker reduces the background 2 to 2.5 fold while decreasing the total number of clones produced per microgram target DNA by 30%. The application discloses that a background of about 1% would be optimal when generating a randomized gene library (page 11, lines 25-28), and Applicants have shown that, unexpectedly, the method of the present invention yields a

background of 1% or less, e.g., 0.5% and 0.3% (page 7, lines 2-4, page 19, lines 29-30 and page 20, lines 30-35), which is improved over prior art methods (page 20, lines 30-35).

The cited art did not recognize that background is a problem in random library generation and neither discloses nor suggests methods to arrive at such efficiency or decreased background. On the contrary, the cited art suggests use of cell lines or methods that would increase background compared to the present method. Butler discloses expressing the γ subunit in γ-sensitive cells, which have a high reversion/mutation rate such that cells are no longer sensitive to the γ toxin. Use of these cells would dramatically increase background detection of positive clones in an expression library. Meinhardt did not recognize that background is a problem in library generation, and could not have proposed methods for improving library construction by reducing background interference in clone selection. The '271 patent suggests that use of a method exhibiting up to 10% background is acceptable for generation of randomized gene libraries, and does not suggest a need to improve the background or methods to improve this background. One of ordinary skill generating a random library would recognize that the high background in the '271 patent was not acceptable (See page 11, lines 26-34 of the specification).

Applicants, in contrast, recognized that background was a problem in creating randomized gene libraries and discovered a that a negative selection cloning method, particularly using the γ subunit, significantly reduced background and improved efficiency over methods in the art (see page 7, lines 2-4, page 19, lines 29-30 and page 20, lines 30-35). Thus, applicants have demonstrated unexpected advantages in the use of the claimed method which were not recognized as a problem in the art.

For the foregoing reasons, the rejection of claims 1-7, 13-17, 24, 27 and 31-32 under 35 USC §103(a) as obvious in view of Butler, Meinhardt and the '271 patent should be withdrawn.

IV. The rejection of claims 22 and 23 under 35 USC §103(a) as assertedly obvious in view of Meinhardt, Butler, US 6,410,271 and Monschau should be withdrawn

The Examiner further rejected claims 22 and 23 as obvious in view of Meinhardt, Butler, the '271 patent and Monschau.

Claims 22 and 23 depend from claim 4, which is patentable over the cited art for the reasons set out above. Monschau does not remedy the deficiencies in the art. Monschau describes use of the TEF promoter from *A. gossypii*, and neither discloses nor suggests use of the γ toxin gene as a negative selection marker. Thus, the rejection of claims 22 and 23 should be withdrawn for the reasons set out above.

V. The rejection of claim 28 under 35 USC §103(a) as assertedly obvious in view of Meinhardt, Butler, US 6,410,271 and Jirholt should be withdrawn

The Examiner further rejected claim 28 as obvious in view of Meinhardt, Butler, the '271 patent and Jirholt.

Claim 28 depends from claim 15, which is patentable over the cited art for the reasons set out above. Jirholt does not remedy the deficiencies in the art. Jirholt discloses a recombination library encoding CDR sequences, but neither discloses nor suggests use of the γ toxin gene as a negative selection marker. Thus, the rejection of claim 28 should be withdrawn for the reasons discussed above.

VI. Conclusion

Applicants submit that the application is in condition for allowance and respectfully request notice of the same.

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